

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: James A. Thomson

Serial No.: 08/591,246

Group Art Unit: 1815

Filed: January 18, 1996

Examiner: B. Brumback

For: PRIMATE EMBRYONIC STEM CELLS File No.: 960296.93723

DECLARATION UNDER 37 C.F.R. §1.132

I, James A. Thomson, on oath do hereby state and declare as follows:

1. I am the same James A. Thomson who is the inventor of the above-identified patent application and I make this Declaration in support of that patent application.

2. The work described in this patent application is directed to the creation of primate embryonic stem cell lines. A procedure for creating such primate embryonic stem cell lines is described in this patent application. Since the filing of this patent application, I have continued to create new primate embryonic stem cell lines using the procedures described in this patent application and have continued to study and characterize the primate embryonic stem cell lines so created. This Declaration will describe some of that work.

3. In continuing to investigate embryonic stem cell lines of rhesus monkey, in my laboratory we have created at least eleven independent lines (including the original one) by the procedure described in the patent application. All the lines we have created are morphologically identical to the cell line designated 278.5 and described in the patent

application here. We have continued to test cell lines for various of the attributes described and claimed in this patent application, but have not tested every cell line for every criteria. We have tested the karyotype for eight of the cell lines. All the karyotypes were normal. Four lines had two X chromosomes and four had one X chromosome and one Y chromosome. I have tested eight of these cell lines in teratomas and the range of differentiation observed was indistinguishable from that observed with R278.5, including differentiation into derivatives of endoderm, mesoderm, and ectoderm tissues throughout the culture. None of the cell lines have been observed to go into senescence. While the first cell lines were extensively tested, the succeeding cell lines have not been tested for a variety of cell surface markers, but for the tests we have conducted on these cell lines of the various cell surface antigens described and claimed in the application, not one negative result has been obtained from the cell lines matching the morphological features of 278.5. The morphology of the cell lines has been distinct and consistent and we have relied on that for identification. In other words, every indication we have so far is that all eleven cell lines meet all of the criteria described and claimed in the claims of the present application for the primate embryonic stem cell lines.

4. In our parallel work being conducted with marmoset monkey cell lines, we have created at least eight such marmoset embryonic stem cell lines. We have tested one of these cell lines to demonstrate the capability to

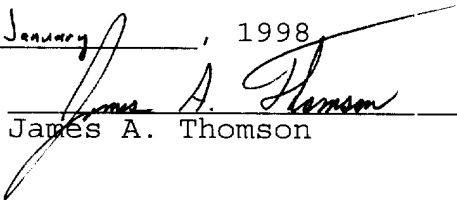
differentiate into all three germ line layers, endoderm, mesoderm, and ectoderm tissues. These lines also have a distinct characteristic that assists in identifying them. The line designated CJ11 described in the patent application has been passaged and maintained for over a year. Other cell lines have not been maintained for as much as a year, as of yet, but none of the cell lines has senesced in any way that would indicate that it is incapable of culture for a year. The cell lines have normal karyotypes, including both XX and XY karyotypes. Again, not every cell line has been tested for all of the cell surface antigens described in this patent application, but every test that has been conducted has been positive for the criteria described in the claims of this patent application. In other words, our data viewed as a whole indicates that it is reliably possible to make numerous primate embryonic stem cell lines in marmoset using the procedures described in the patent application, and the cell lines would meet the criteria claimed in the present patent application for those cell lines.

5. These data demonstrate that the method described in this patent application can be used reliably and reproducibly, and without undue experimentation, to create primate embryonic stem cell lines in culture. While the work is arduous and technical, the procedures for performing the work are described in the patent application in sufficient detail to enable reproduction of this procedure by those of skill in this art.

6. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further,

that these statements are made with the knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under Section 1001, Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated this 30 day of January, 1998


James A. Thomson

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